A Hypothetic Mechanism of Ordering and Shaping Cells within Discrete Regions Created by Descendants of AB Blastomere During Early Stages of C. Elegans Embryogenesis. A Theoretical Study.

Yaroslav N. Strokovskyy
yaroslav-strokovskyy@yandex.ru

Abstract.
We propose a hypothetic mechanism of ordering and shaping cells during C.elegans embryogenesis. The mechanism is based on the assumption that during each round of division of cells, daughter cells acquire electric charges from its mother cells (charge conservation law is taken into account). Between the acts of division, the total electric field, generated by a system of charged cells, change relative positions of the charged cells within the region. In one's turn, the positions of the charged cells determine the pattern of the electrostatic field. A mathematical model of such self-consistent mechanism is developed. The process of developing the sixteen-cell structure starting with one cell is simulated. The results correspond to the known experimental data.

Introduction.

C. elegans embryo achieves pattern formation by sorting cells, through far-ranging movements, into discrete regions before morphogenesis is initiated [1]. A proposed mechanism [1] of the sorting process is described in a model for guidance by "cell focusing". According to this model, a certain "positional value", which is generated on a cell's surface, guides cells to their proper position within the discrete region. In this model, forces that ought to guide the cells during embryonic development are not defined explicitly and thus, any numerical estimations impossible. The model may be regarded as descriptive.

In this study, a mathematical model of the sorting process is developed. Using the model, the process of sorting is simulated and the results are compared with the known experimental data.

General considerations.

In order to develop a mathematical model, the forces that guide the cells during embryogenesis, must to be defined explicitly. Known experimental data do not indicate directly the origin of the forces. Thus, as for the "cell focusing" model, assumption on this issue must to be done. Correctness of the assumption may be checked by comparing the results of simulation and experimental data.

According to [1], [2], the following features are essential for the development process 1) descendants of the 8-AB blastomeres form discrete regions in the embryo body plan, 2) within the discrete region, descendants of cells form sub regions, 3) migrations (including long-range migrations) of cells serve to order the regions with respect to each other and to shape the forms of the regions, 4) a mechanism of ordering and shaping must function in a constantly changing environment.

Let us modernize the "cell focusing" model and suppose that the electrical charge represents the "positional value" and thus, Coulomb's law ($F \sim Q^2/r^2$) directly determines the physical forces that guide the cells within the region.

In such regions, each cell changes its position due to the pattern of the total electrostatic field that is generated by all other cells. In one's turn, the pattern of the total electrostatic field in the region depends on the position of each cell. Thus, migrations of the cells change the pattern of the electrostatic field and, in one's turn, the new pattern defines the new positions of the cells. Described system may be defined as self-configuring. In such system, the migrations of the cells

Nature Precedings : doi:10.1038/npre.2010.4277.1 : Posted 11 Mar 2010

occur as long as the system will not be in a potential well (kinetic energy dissipation is not equal to zero). The question arises: Can a system of charged cells create compact and stable conglomerate (region)? Although the final positions of cells can be determined by solving the corresponding so-called self-consistent equations (see below), some features of the self-configuring system can be determined by taking into account general considerations, and thus, one can easily assess the capabilities of the proposed model. Let us analyze some examples.

1. Two cells with opposite charges represent primitive compact and stable conglomerate. In this case, the cells are attracted each other and keep moving until a collision. After the collision, the forces of electrical attraction is compensated by the reaction of the body of the cell. The system reaches a potential well. In the potential well, random exposure, which alter the position of the cell, give rise to a focusing force and the cell returns back into the potential well. Such compact and stable self-configuring system may be regarded as a self-focusing system.

2. Let us analyze a 2-D discrete region, which consists of N>1 cells with charges of the like signs (q_i), and one cell with the charge of opposite sign (Q). The position of the cells (a shape of the discrete region) depends on value of charges q_i and Q. If the region as a whole is neutral ($\Sigma q_i+Q=0$) and the value q_i of all the charges cells are identical ($q_i=q0$, $i=1,2,\ldots N$, hereinafter, the cells are marked in accordance with the magnitude of their charge), then the discrete region acquires the shape of a circle with the cell Q in the center of the circle. The displacement of any q_i cell outside the electrically neutral region (circle) give rise a focusing effect, which returns the cell back to the region. In this case, the region maintains its integrity from random external influences and may be regarded as a stable, self-focusing system. Displacement of any cell q_i inside the region does not alter the pattern of the electrostatic field and, thus, within the region, the effects of sorting and focusing do not occur.

3. If within the above-described discrete region, there are several groups of cells with the same magnitude of charge in each group, then, these groups will be sorted in several concentric circles. Each circle contains cells with the same value of charge. The more modulus of the value of charge, the closer the group is located to the central cell Q. As in previous case, the region maintains its integrity from random external influences and may be regarded as a stable, self-focusing system. The displacement of any q_i cell outside its native group changes the pattern of the electrostatic field and give raise a focusing effect, which returns the cell back to the group. Thus, groups maintain their integrity and may be regarded as self-focusing systems. The displacement of cells within the group does not alter the pattern of the electrostatic field and, thus, within the group, the effects of sorting and focusing do not occur.

4. If in the above discrete region, there are no cells with identical values of charges (q_i) then, one can assume that the discrete region will have an asymmetrical shape. In this case, the placement of cells is not clear. However, it can be argued that the greater $|q_i|$, the closer this cell is located to the Q cell (because the interaction force is greater). As in previous cases, the region maintains its integrity and may be regarded as self-focusing system. The displacement of the cells within the discrete region alters the electrostatic pattern of the region (because values of charges are different) and thus, interaction forces appear. However, whether these forces are able to provide focusing effect is not clear.

The above-mentioned primitive examples demonstrate that a system of charged cells can create compact and stable conglomerate (region). The distribution of charges between the cells determines the position of cells and shape of the discrete region. Described below is a mathematical model allows us to analyze the sorting process for the arbitrary distribution of charge between cells

A mathematical model.

In this model, a point charge represents a cell with a radius of R_{cell}.

In order to obtain both the magnitude and direction of the force on a cell, with a charge q_i at position $\vec{R}_i$, experiencing a field due to the presence of another cell with a charge q_j at position $\vec{R}_j$, the full vector form of Coulomb's law is used.

$$\vec{Fc}_{ij} = \frac{q_i}{4\pi\varepsilon_0}\frac{q_j}{R_{i,j}^2}\vec{r}_{i,j}$$

where R_{ij} is the separation of the centers of the two cells

$\vec{r}_{i,j}$ is a unit vector parallel with the line from charge q_i to charge q_j.

ε_0 is the electric constant

The principle of liner superposition is used to calculate the force on a cell with a charge q_i at position R_i, due to a system of N cells with charges:

$$\vec{Fc}_i = \frac{q_i}{4\pi\varepsilon_0}\sum_{\substack{j=0\\ j\neq i}}^{N}\frac{q_j}{R_{i,j}^2}\vec{r}_{i,j}$$

According to Newton's second law the force $\vec{Fn}_i$ applied to a cell produces a proportional acceleration $\frac{d\vec{V}_i}{dt}$. The relationship between the two is

$$\vec{Fn}_i = m\frac{d\vec{V}_i}{dt} \qquad \vec{V}_i = \frac{d\vec{R}_i}{dt}$$, where m is a mass of a cell, t is time.

In order to model a body of a cell, i.e. to limit minimum separation between cells (approximately, to $2R_{cell}$), an auxiliary function is used:

$$\vec{Faxu}_i = 4kR_{cell}^2\frac{|q_i|}{4\pi\varepsilon_0}\sum_{\substack{j=0\\ j\neq i}}^{N}\frac{|q_j|}{R_{i,j}^4}\vec{r}_{i,j}$$, $k\approx 1$ is a factor.

At great separation ($R_{i,j} \gg R_{cell}$), this function provides week repulsion effect, at $R_{i,j} < R_{cell}$ the repulsion force (F_{aux}) exceed attraction force (F_c), and thus, the separation increases.

Equating $\vec{Fn}_i$ and $\vec{Fc}_i + \vec{Faux}_i$, a system of self-consistent equations appears:

$$m\frac{d\vec{V}_i}{dt} = \frac{q_i}{4\pi\varepsilon_0}\sum_{\substack{j=0\\ j\neq i}}^{N}\frac{q_j}{R_{i,j}^2}\vec{r}_{i,j} + 4kR_{cell}^2\frac{|q_i|}{4\pi\varepsilon_0}\sum_{\substack{j=0\\ j\neq i}}^{N}\frac{|q_j|}{R_{i,j}^4}\vec{r}_{i,j}$$

$$\vec{V}_i = \frac{d\vec{R}_i}{dt} \qquad i = 0,1,2,.....N$$

The initial conditions at $t=t_0$ are defined as $\vec{V}_i = \vec{V}_{i0}$ $\quad \vec{R}_i = \vec{R}_{i0}$

The equations, together with the initial conditions, represent the mathematical model of the self-configuration (sorting) process under the assumption that charged cells represent "positional value". Solution of the equations shows the spatial position of the charged cells (configuration of cells) over the time.

Numeric solution.

Let us use the above-mentioned equations to simulate the process of creating a two-dimensional discrete region starting with one cell. In order to do this we take into account the following considerations.

- Value of charge of the founder cell is equal to Q.
- The development consists of a series of successive rounds. Each round consists of mitosis and the following process of the self-configuring (sorting).
- After mitosis, during a certain period, the cells self-configure itself in accordance with their values of charge. The self-consistent equations model the process of configuring (sorting). In this study, the process of self-configuring terminates when positions of the cells cease to change with time (steady-state configuration, in this state, the system of the charged cells is located in a potential well). This requirement is chosen to simplify calculations. The model allows simulation for arbitrary periods between acts of division of each cell. In this case, unsteady-state configuration defines the initial conditions for the next round of self-configuring process.
- The spatial position of cells at the end of each self-configuring process determines the initial conditions for the next round in accordance with the following "initial" rule. Center of inertia of the two daughter cells coincides with the center of inertia of the mother cell. The angle of division is 45 degrees with respect to alleged a-p axis. Daughter cells acquire charges from her mother in accordance with the following "charge" rule: $q_{daughter1}=\alpha q_{mother}$ and $q_{daughter2}=(1-\alpha)q_{mother}$. The rule is consistent with charge conservation law $q_{daughter1}+q_{daughter2}=q_{mother}$. The rule is applied to all cells within region and in each round. This implies that the internal mechanism of cells, which provides (if so) this operation, may be the same in each cell.
- The self-consistent equations have been transformed into a convenient form for programming, normalized, and programmed. The common fourth-order Runge-Kutta method was used to obtain numeric solution.
- Normalized value of the charge of the founder cell is chosen equal to 5. Parameter α is chosen equal to 1.25. In order to avoid bouncing of cells, attenuation of speed of cells is chosen to 0.001.

In the first round, the founder cell X divides and two daughter cells Xa and Xp are involved in the self-configuring process. Under the "charge" rule, the cells acquire charges of opposite signs and thus, the dipole must represent the final configuration. Fig. 1 shows the numeric solution of the self-consistent equations at the end of the first round, the inserted picture shows the position of the cells at the beginning of the round. The dipole represents configuration of two charges. The numbers show the value of the charge that the cells acquire in accordance with the "charge" rule. The total charge of the system is equal to the charge of the founder cell X. Identification of cells is in accordance with their linage

In the second round, four cells are involved in the self-configuring process. Fig 2 shows the numeric solution of the self-consistent equations at the end of the second round, the inserted picture shows the position of the cells at the beginning of the round. The numbers show the value of the charge that the cells acquire in accordance with the "charge" rule. The total charge of the system is equal to the charge of the founder cell. The total charge of each newborn pair is equal to the charge of their mother cell. Identification of cells is in accordance with their linage Diamond represents the final configuration. With respect to the d-d' line, the cells occupy positions that can be considered as a mirror symmetric. Symmetrically located cells have the same charges.

In the third round, eight cells are involved in the self-configuring process. Fig 3 shows the numeric solution of the self-consistent equations at the end of the second round, the inserted picture shows the position of the cells at the beginning of the round. The numbers show the value of the charge that the cells acquire in accordance with the "charge" rule. The total charge of the system is equal to the charge of the founder cell. The total charge of each newborn pair is equal to the charge of their mother cell. Identification of cells is in accordance with their linage. With respect to the d-d' line, the cells occupy positions that may be regarded as a mirror symmetric. Symmetrically located cells have the same charges.

In the forth round, sixteen cells are involved in the self-configuring process Fig 3 shows the numeric solution of the self-consistent equations at the end of the second round, the inserted picture shows the position of the cells at the beginning of the round. The numbers show the value of the charge that the cells acquire in accordance with the “charge” rule. The total charge of the system is equal to the charge of the founder cell. The total charge of each newborn pair is equal to the charge of their mother cell. Identification of cells is in accordance with their linage. Except for the Xpaaa cell, others take positions that may be considered as a mirror symmetrical position, although slightly distorted. The charge of the Xpaaa cell is the cause of the distortion. With respect to the d-d’ line, the cells with identical charges occupy a mirror symmetrical positions.

Discussion.

Let us pay attention on the following. In accordance with Fig 1-Fig 4:

1) The founder cell X creates discrete regions at each round.

2) In the second and following rounds, Xa cell and Xp cell create their own sub regions (red and yellow spheres designate Xa sub region, green and violet spheres designate Xp sub region). At the third and following rounds Xaa cell (red), Xap cell (yellow), Xpa (green) and Xpp (violet) create their own sub sub regions.

3) In each round, the initial and final positions of the cells are different. Thus, the migration of cells affects the shape of the region (sub regions).

4) In each round, after a certain period, the positions of cells cease to vary with time (steady-state configuration). In this state, the system of the charged cells located in the potential well and, thus, each cell can withstand external influences.

Taking into account the above, we conclude that the simulated structure corresponds to the requirements that are essential for the development process (see General considerations).

What is essential for the creation of such self-focusing systems? One can see that if the factor $\alpha<1$, then, according to the “charge” rule ($q_{daughter1}=\alpha q_{mother}$ and $q_{daughter2}=(1-\alpha)q_{mother}$), all descendants of the founder cell acquire charges of the same sign. In such system, all the cells repel each other, the separations between the cells have increased steadily over time and, consequently, the system expands (at least in the free space). In this case, the region may be considered only as self-configuring. The effect of the self- focusing does not occur in this region. Thus, in order to form a compact region, the factor α must be greater then 1. In this case, in accordance with the “charge” rule, cells with charges of opposite signs appear. Exactly the interaction between charged cells of opposite signs ensures the formation of the region, which can be regarded as self-configuring and self-focusing.

Summarizing, it can be argued that the superposition of long-rang force fields generated by each cell of the region provides a process of self-configuring. There are three classic long-range force fields: electrical, magnetic and gravitational fields. Gravity provides the weak interaction and thus, the electrical field, magnetic field, or both may be considered as possible agents in the process of self-configuring.

In order to ensure the distribution of charges between the daughter cells, mother cells must have an appropriate internal mechanism (say, electrochemical). In this study, the internal mechanisms of the cells are not analyzed. Nevertheless, it may be argued that the relevant electrochemical mechanisms exist, at least among adult living beings (cramp-fish is an example). Thus, the existence of similar mechanisms in the cells of the embryo can be regarded as more or less likely.

According to the model, the charged cells are arranged in mirror-symmetrical order and create a complicated symmetrical pattern of the electrostatic field. From the engineering point of view, the cells can use such field as a coordinate system to enable (disable) the corresponding mechanism in the cell according to the level of the electrostatic field, i.e to cause the process of cell differentiation. Thus, the symmetrical components of the cellular structure may acquire and symmetrical functional properties.

References.
1. Schnabel, R., Hutter, H., Moerman, D., Schnabel, H., 1997. Assessing normal embryogenesis in Caenorhabditis elegans using a 4D microscope: variability of development and regional specification. Dev. Biol. 184 (1997), 234–265.
2. Ralf Schnabel, Marcus Bischoff, Arend Hintze, Anja-Kristina Schulz, Andreas Hejnol, Hans Meinhardt, Harald Hutter. Global cell sorting in the C. elegans embryo defines a new mechanism for pattern formation. Dev. Biol. 294 (2006) 418–431

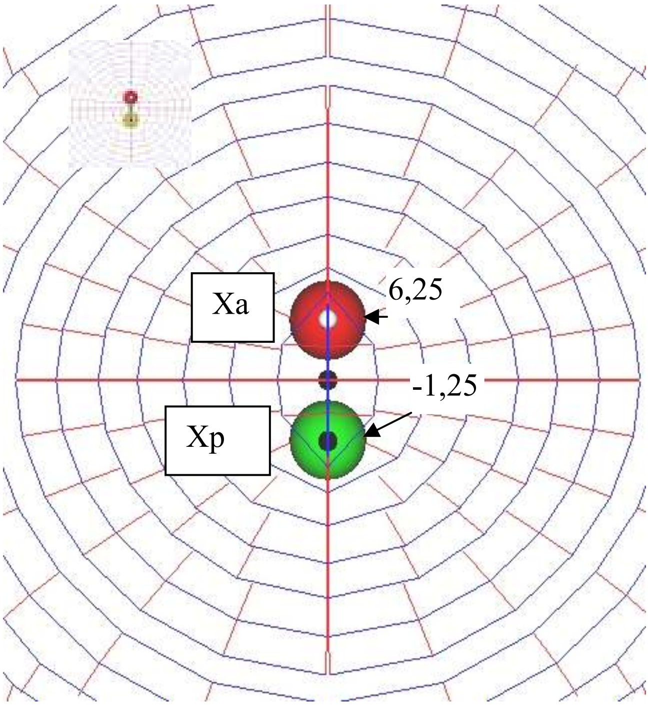

Fig. 1. The final configuration of two charged cells at the end of the first round of the process of self-configuring.
The numbers show the value of the charge that the cells acquire in accordance with the “charge” rule.
The total charge of the system is equal to the charge of the founder cell. Identification of cells is in accordance with their linage.
Dipole represents the final configuration.

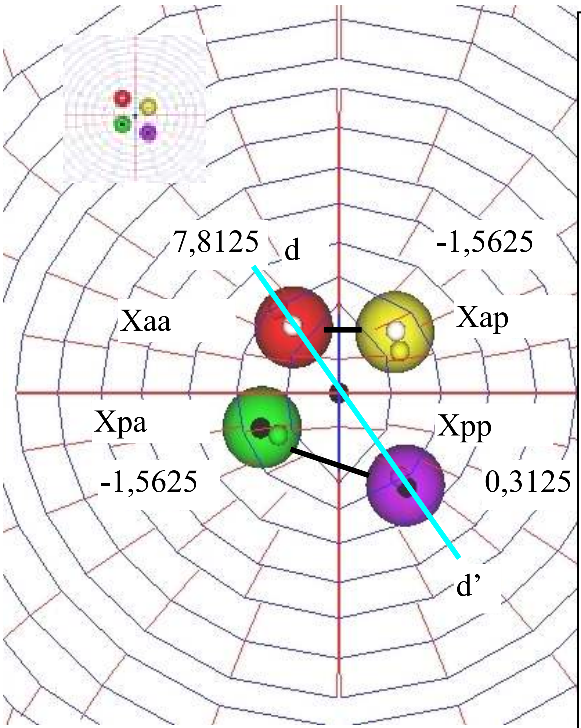

Fig. 2. The final configuration of four charged cells at the end of the second round of the process of self-configuring. The numbers show the value of the charge that the cells acquire in accordance with the “charge” rule. The total charge of the system is equal to charge of the founder cell. The total charge of each newborn pair is equal to the charge of their mother cell. Identification of cells is in accordance with their linage.

Diamond represents the final configuration. With respect to the d-d’ line, the cells occupy positions that can be considered as a mirror symmetric. Symmetrically located cells have the same charges.
Inserted picture represents the position of cells at the beginning of the round of self-configuring process.

.

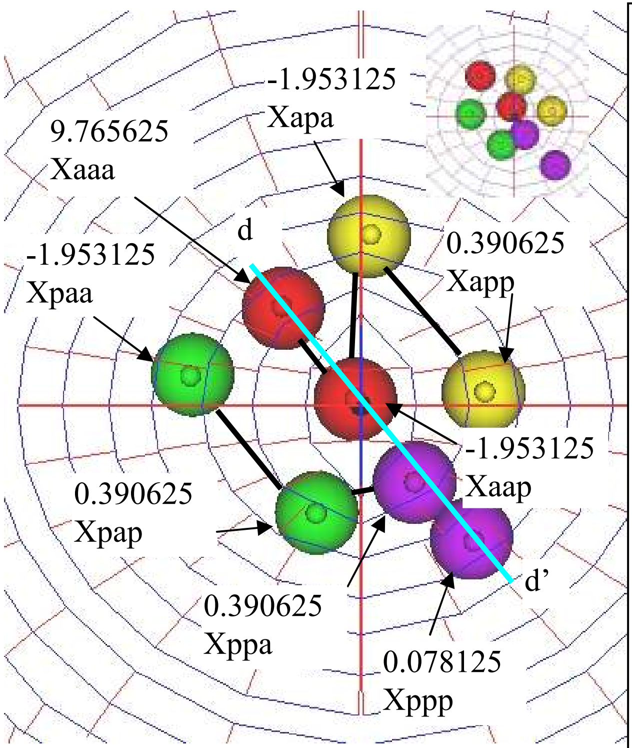

Fig. 3. The final configuration of eight charged cells at the end of the third round of the process of self-configuring. The numbers show the value of the charge that the cells acquire in accordance with the "charge" rule. The total charge of the system is equal to charge of the founder cell. The total charge of each newborn pair is equal to the charge of their mother cell. Identification of cells is in accordance with their linage.

With respect to the d-d' line, the cells occupy positions that may be regarded as a mirror symmetric. Symmetrically located cells have the same charges.
Inserted picture represents the position of cells at the beginning of the round of self-configuring process.

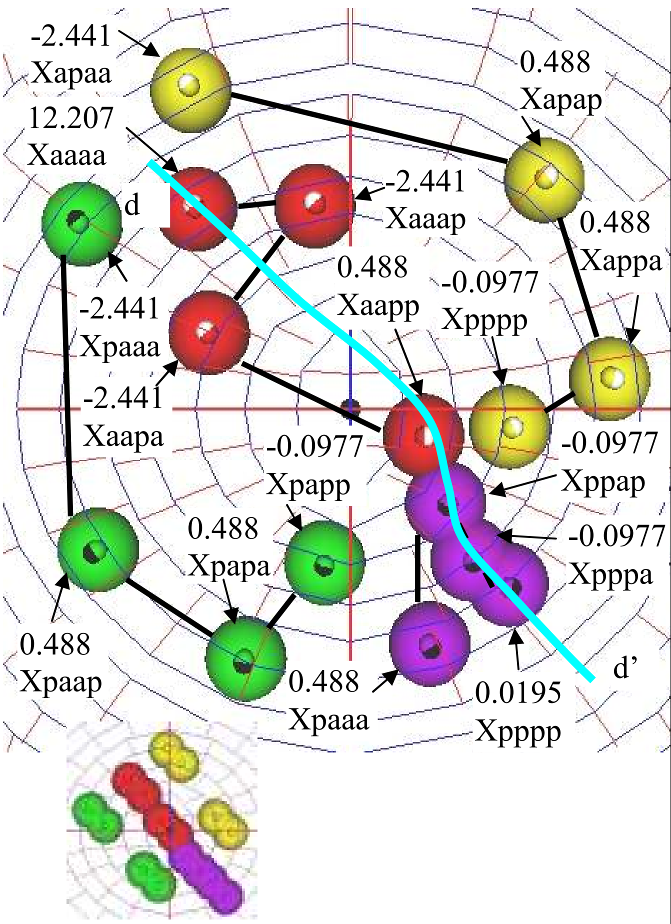

Fig. 4. The final configuration of sixteen charged cells at the end of the forth round of the process of self-configuring. The numbers show the value of the charge that the cells acquire in accordance with the "charge" rule. The total charge of the system is equal to charge of the founder cell. The total charge of each newborn pair is equal to the charge of their mother cell. Identification of cells is in accordance with their linage.

Except for the Xpaaa cell, others take positions that may be considered as a mirror symmetrical position, although slightly distorted. The charge of the Xpaaa cell is the cause of the distortion. With respect to the d-d' line, the cells with identical charges occupy a mirror symmetrical positions.
Inserted picture represents the position of cells at the beginning of the round of self-configuring process.